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Rabenstein M, Shin YK.

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#415 Enhanced Immunogenicity to HIV-1 Envelope Using DNA Vaccines

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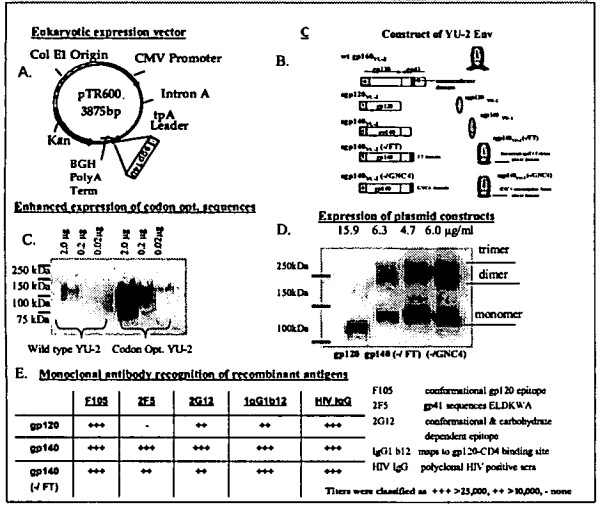
Background & Purpose

Recent studies suggest that HIV-1 envelope (Env) is a trimer on the native virus particle. In addition, it has been proposed that oligomeric/trimeric envelope vaccines may elicit better neutralizing antibodies because they more closely mimic the native conformation of gp160 Env. Two soluble, trimeric HIV-1 YU-2 envelopes (sgp140), each stabilized by different trimeric motifs; one from the GNC4 transcription factor and the other from bacteriophage T4 fibritin, have been shown to mimic the native oligomeric, transmembrane-bound gp160 Env. Both sequences are codon optimized for enhanced expression and have been shown to efficiently from and secreted trimers from cells. The proteolytic cleavage site, at the gp120/gp41 junction of each molecule, has been modified to prevent cleavage of the two subunits.

The goals of this study were:

- To compare the immunogenicity of soluble, stabilized trimeric forms of Env to unstabilized sgp140 and sgp120 versions with three vaccination regimens.
- To assess the role of C3d 1) to act as a molecular adjuvant to enhance the immunogenicity and affinity maturation of antibodies, 2) on cell mediated responses.

Figure 1: Construction and Expression of Vaccine Plasmids



E. Monoclonal antibody recognition of recombinant antigens

	F105	2F5	2G12	1gG1b12	HIV IgG
gp120	+++	-	++	++	+++
gp140	+++	+++	+++	+++	+++
gp140 (-FT)	+++	++	++	+++	+++

F105 conformational gp120 epitope
2F5 gp41 sequences ELDKWA
2G12 conformational & carbohydrate dependent epitope
IgG1 b12 maps to gp120-CD4 binding site
HIV IgG polyclonal HIV positive sera

Titers were classified as +++ >25,000, ++ >10,000, - none

Figure 1. All constructs efficiently express in-vitro and both GNC4 and FT stabilized versions of sgp140 form trimers after boiling and denaturing conditions (D). Expression of codon optimized DNA sequences is greatly enhanced compared to wildtype (C). Purified recombinant antigens used for protein inoculations recognized a panel of monoclonal antibodies (E).

Figure 2: Immune Response

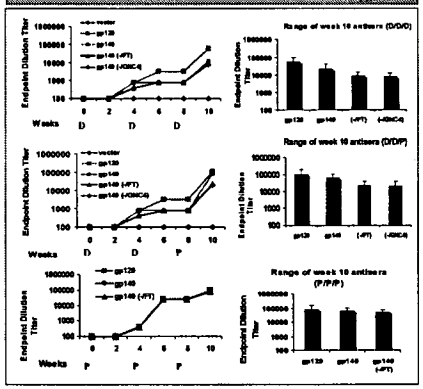


Figure 2. BALB/c mice (n=5)were primed at day 0 with either 2µg DNA (D) by genegun or inoculated IP with recombinant protein (P), and subsequently boosted at weeks 4 and 8 as indicated. Sera, collected at week 10 were analyzed for anti-Env specific antibodies. Similar titers of total IgG anti-Env antibodies were observed in mice vaccinated with DNA expressing any of the envelope proteins, regardless of the regimen used to inoculate the mice. In addition, the total anti-Env IgG antibody titers were similar in mice vaccinated with DNA or recombinant protein.

Table 1: Antibody: Antigen Recognition

	D/D/D (Total IgG Ab Titer 1X 10^3)				D/D/P (Total IgG Ab Titer 1X 10^3)				P/P/P (Total IgG Ab Titer 1X 10^3)		
Coating Antigen	gp120	gp140	(-FT)	(-GNC4)	gp120	gp140	(-FT)	(-GNC4)	gp120	gp140	(-FT)
gp120	0.78	0.12	0.09	0.08	1.04	0.83	0.24	0.16	0.83	0.68	0.67
gp140	0.77	0.58	0.28	0.26	1.28	2.40	1.28	1.02	0.77	.46	0.54
gp140 (-FT)	0.77	0.51	0.29	0.29	1.28	2.05	1.22	0.64	0.65	.40	0.45

Table 1: Ten week sera from mice vaccinated with, $sgp140_{YU-2}$, $sgp140_{YU-2}$(-/FT), and $sgp140_{YU-2}$(-/GNC4) via DNA prime and either DNA or protein boost (D/D/D or D/D/P) strategy had approx. 3-6 fold higher Ab titers when coated against $gp140_{YU-2}$ or $gp140_{YU-2}$ (-/ FT) antigens than against $gp120_{YU-2}$ antigens . Mice vaccinated with gp140 or gp140(-/ FT) recombinant protein (P/P/P) had similar titers regardless of the antigen coated. Similarly, mice vaccinated with gp120 via any of the three regimens all had similar Ab titers.

C3d as a molecular adjuvant

Previous work from our laboratory has shown:

- 3 copies of murine C3d (mC3d3) fused to HIV-1 envelope and hemagglutinin (HA) of measles and influenza can act as a molecular adjuvant and enhance immunogenicity and affinity maturation of antibodies.
- sHAmC3d3 can afford protect against virus challenge with ten times less DNA than sHA alone; and plays a role in shaping the cell-mediated response with a Th2 bias.

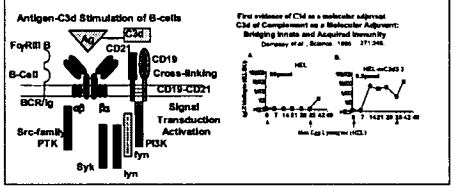


Figure 3: Expression & Immunogenicity of Env-C3d

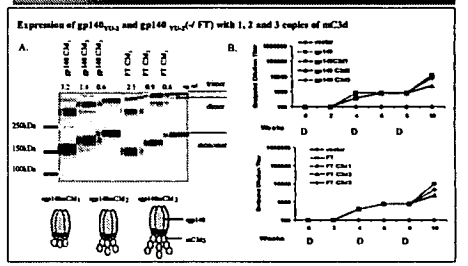


Figure 3. DNA vaccine plasmids encoding Env-C3d fusions were constructed and tested for expression; even after the addition of C3d, sgp140(-/FT) trimers were stable (A). Mice were vaccinated as described in Figure 2 (B). All codon-optimized sequences had enhanced Env-specific Ab titer, regardless of the addition of C3d.

Table 2: ELISpot

	# spots (10x6 cells) IL-4				# spots (10x6 cells) INF-γ			
	P1	P6	P15/16	Env	P1	P6	P15/16	Env
(-/FT)	43	31	9	8	24	13	7	16
(-/FT) $C3d_1$	43	28	9	17	19	15	3	21
(-/FT) $C3d_2$	64	37	9	11	43	17	13	19
(-/FT) $C3d_3$	88	67	7	13	63	17	8	9

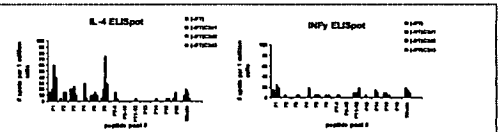


Table 2: Mice (n=3), described in Figure 3 were given a 4th inoculation of DNA at week 28, and spleens were harvested 10 days later. Splenocytes were prepared and $1x10^6$ cells/ml were re-stimulated with peptide pools from HIV-1 MN Env at 10µg/ml, or with 1µg/ml recombinant protein. For ELISpot assays, splenocytes were stimulated overnight and added to pre-coated plates (R&D Systems). Figures represent responses against the complete panel of HIV-1 MN corresponding to the entire gp140 portion of Env in pools of 18 peptides.

Table 3. Proliferation

	^{3}H-thymidine incorporation CPM per 10x6 cells			
	P1	P6	P16	Env
(-/FT)	379	599	-	278
(-/FT) $C3d_1$	1252	869	37	542
(-/FT) $C3d_2$	983	1009	-	1240
(-/FT) $C3d_3$	1127	1153	-	464

Table 3: Mice (n=3), as described in Figure 3, were given a 4th inoculation of DNA at week 28, and spleens were harvested 10 days later. Splenocytes were prepared and $1x10^6$ cells/ml were re-stimulated with peptide pools (10 peptides per pool) from HIV-1 MN Env at 10µg/ml, or with 1µg/ml recombinant protein. For proliferation, splenocytes were stimulated for 4 days, and ^{3}H-tymidine was added and cells harvested 48 hours later.

SUMMARY

- The results of this study show that codon-optimized DNA vaccines expressing various Envs elicit similar, high levels of anti-Env Abs, as protein inoculations.
- Codon-optimized sequences blunt the effects of C3d, in its ability to enhance the immunogenicity of Env. However, C3d plays a role in enhancing a balanced T helper cell response.

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